American Journal of Medical Genetics 69:85–88 (1997)

Use of Stored Newborn Blood Spots in Research on Birth Defects: Variation in Retrieval Rates by Type of Defect and Infant Characteristics

Christopher A. Loffredo[1*] and Cynthia K. Ewing[2]

[1]*Department of Epidemiology and Preventive Medicine, University of Maryland School of Medicine, Baltimore*
[2]*The Center for Medical Genetics, Johns Hopkins University School of Medicine, Baltimore, Maryland*

Stored blood spots from state newborn screening programs represent a potential source of DNA for molecular genetics research on birth defects. The stored blood spots of cases and controls from an epidemiologic database on congenital heart defects were sought in the present study, which aimed to establish the feasibility of linking the data sources and to examine blood spot retrieval rates for selected cardiac defects. Blood spots were located on 341 of 522 infants (65%) with congenital heart defects and for 1,484 of 1,645 infants without birth defects (84%) born in Maryland during 1981–1989. Retrieval rates were low among infants with clinically severe lesions such as truncus arteriosus (26%) but were considerably higher in infants with coarctation of the aorta (62%), pulmonic valve stenosis (71%), and atrial septal defect (76%). Retrieval rates were significantly lower for premature and low–birth-weight infants than among full-term, normal–birth-weight infants. Retrieval rates did not vary significantly by gender, race, county of residence, or parental socioeconomic characteristics. These results demonstrate the feasibility of linking epidemiologic databases with stored newborn blood specimens, especially for normal infants and even for infants with certain congenital heart defects, but raise concerns about the adequacy of such methods to obtain stored specimens from premature infants and from those with severe heart defects. Am. J. Med. Genet. 69:85–88, 1997. © 1997 Wiley-Liss, Inc.

KEY WORDS: blood spots; congenital heart disease; newborn screening

INTRODUCTION

Newborn screening programs for hereditary metabolic disorders such as phenylketonuria routinely collect blood spots on filter paper blotters ("Guthrie cards"). In many states the dried blood spots are stored, and current policies in some states permit sharing these samples with third parties for research purposes [McEwen and Reilly, 1994]. The development of a rapid, low-cost laboratory method, polymerase chain reaction (PCR), has made the amplification of DNA extracted from dried blood spots possible [McCabe, 1991]. Testing these DNA samples for genetic variations associated with the occurrence of specific birth defects, childhood cancers, and other diseases is of great interest.

The success of Hwang et al. [1995] in matching records from a birth defects registry with stored blood spots suggested the possibility of building on an existing population-based case–control study of congenital heart defects. In this study, we collected stored blood spots of infants born in Maryland during 1981–1989 with heart defects and a random sample of normal controls as part of a separate project on potential genetic-environmental interactions. The aims of this report are to describe our experience in linking an epidemiologic database with blood samples from a state newborn screening program and to examine factors that may have influenced retrieval rates of blood spots from infants with and without specific congenital heart defects.

MATERIALS AND METHODS

Study Population

The subjects for this study were Maryland-born infants from the Baltimore-Washington Infant Study (BWIS), a population-based case–control study of resident, liveborn infants with congenital heart defects born in Maryland, the District of Columbia, and north-

*Correspondence to: Christopher A. Loffredo, Department of Epidemiology and Preventive Medicine, University of Maryland, 660 West Redwood Street, Baltimore, MD 21201.

Received 28 March 1996; Accepted 17 June 1996

ern Virginia during 1981–1989. BWIS cases (N = 4,390) were infants diagnosed by pediatric cardiologists during the first year of life, with diagnostic confirmation by echocardiography, cardiac catheterization, surgery, or autopsy. BWIS controls (N = 3,572) were a representative sample of liveborn infants without congenital heart defects who were randomly selected from the area birth hospitals in proportion to each hospital's contribution to the total regional live birth cohort. Parents of cases and controls were interviewed concerning medical and sociodemographic factors and exposures to potential environmental risk factors. A detailed description of BWIS methods has been published [Ferencz et al., 1993].

As part of our separate study testing for associations between markers in a series of candidate genes and specific cardiac defects, we targeted the following six cardiac diagnoses from the BWIS for retrieval of dried blood spots: pulmonic stenosis, aortic stenosis, coarctation of the aorta, atrial septal defect, truncus arteriosus, and interrupted aortic arch. All BWIS cases with these specific defects born in Maryland during 1981–1989 were eligible for blood spot retrieval; we excluded infants with associated noncardiac anomalies. A random sample of the Maryland-born BWIS controls (approximately three per case) were frequency matched to these cases by year and hospital of birth.

Blood Spot Collection Procedure

Newborn screening in Maryland is voluntary and requires informed parental consent. In addition to a verbal description of the program, parents are given an easily understandable pamphlet describing the benefits of the screening tests and stating that, "From time to time, the state laboratory conducts studies on other important health problems using these specimens. These are done only after all the other tests are completed . . . these studies do not tell us whether individual babies have the problem being studied, because the samples are not identified by name."

A two-step process was required to locate the blood spots stored at the Newborn Screening Program of Maryland's Department of Health and Mental Hygiene (DHMH), following approval by the DHMH review board, the review boards of the University of Maryland and Johns Hopkins University, and the BWIS coinvestigators. The collection procedure was performed with case–control status masked and was undertaken between August 1994 and March 1995.

Because each blood spot card carried only a date and unique laboratory number as identifiers, the first step was to match each infant's name, date of birth, and hospital of birth from the BWIS data with the corresponding data on the DHMH lists. This step obtained the laboratory number of the infant's blood spot. The second step was to search boxes of stored blood spots by date and laboratory number to find the actual specimens. From each filter paper card of four or five blood spots, two spots were removed for PCR analysis.

RESULTS

A total of 522 cases and 1,645 controls were targeted for blood spot retrieval, of whom 341 (65%) and 1,484 (84%), respectively, were located successfully. The retrieval rate among cases varied by cardiac diagnosis, ranging from 26% among infants with truncus arteriosus or interrupted aortic arch to 76% among those with atrial septal defect (Table I). The main sources of retrieval failure were lack of a matching laboratory number in the DHMH records, which accounted for most of the retrieval failures, and lack of a documented specimen.

To examine possible sources of bias, we compared the retrieved and nonretrieved subjects on several infant and familial characteristics. We excluded cases with truncus arteriosus and interrupted aortic arch from these comparisons due to their high neonatal mortality rate (70%), which impaired newborn screening. Among the remaining cases, retrieval rates were considerably lower if the infant had a birth weight less than 2,500 g or was born earlier than 38 weeks gestation, relative to infants with normal weight and gestational age (Table II). Cases who had died in the first year of life were less likely to be traced successfully than infants who survived. Among the controls, retrieval rates were also reduced for premature infants and for infants with low birth weights, but the rate differentials were less than those of cases; all controls survived the first year of life. In cases and controls, retrieval rates were not associated with infant race, gender, or county of residence; family income; or maternal age, parity, marital status, and educational level.

DISCUSSION

This descriptive study demonstrates that collection of dried newborn blood spots as a source of DNA for PCR analysis is feasible for infants in Maryland who have congenital heart disease. As this is among the first reports to document this type of sample collection in a

TABLE I. Blood Spot Retrieval Among Cases and Controls Born in Maryland (1981–1989)

Group	Total sought	No DHMH[a] lab number	Lab number but no blood spot	Lab number and blood spot
Cases (total)	522	157 (30%)	24 (5%)	341 (65%)
Pulmonic stenosis	169	38 (22%)	11 (7%)	120 (71%)
Aortic stenosis/coarctation	161	55 (34%)	6 (4%)	100 (62%)
Atrial septal defect	142	28 (20%)	6 (4%)	108 (76%)
Truncus/interrupted arch	50	36 (72%)	1 (2%)	13 (26%)
Controls	1645	161 (10%)	103 (6%)	1381 (84%)

[a]DHMH, Department of Health and Mental Hygiene.

TABLE II. Blood Spot Retrieval Rates by Infant Characteristics

	Blood spots of cases (N = 472)[a]				Blood spots of controls (N = 1645)			
	Retrieved	Not retrieved	Total	Rate	Retrieved	Not retrieved	Total	Rate
Birth weight (g)								
<2500	34	47	81	0.42	65	36	161	0.64
2500+	294	97	391	0.75*	1,316	228	1544	0.85*
Gestational age (wk)								
<38	48	48	96	0.50	116	51	167	0.69
38+	279	96	375	0.74*	1,264	213	1477	0.86*
Vital status at 1 year								
Alive	318	129	447	0.71	1,381	264	1645	0.84
Dead	10	15	25	0.40*	0	0	0	0.00

* $P < 0.001$ (chi-square test; 2 df).
[a] All cases combined, excluding truncus arteriosus and interrupted aortic arch.

study of congenital heart defects, our results may be useful to other researchers planning similar studies.

As technologic advances in molecular genetics have facilitated studies using stored blood spots, some authors have expressed concern about the ethics of the research methods. Recently, the American College of Medical Genetics Storage of Genetics Materials Committee [1995] and a Consensus Workshop Group convened by the National Center for Human Genome Research [Clayton et al., 1995] released policy statements on this issue. The need to remove personal identifiers from existing samples and the need for informed consent were clearly expressed. The parental informed consent aspect of Maryland's newborn screening program addresses the issue of using blood spots for research purposes, and we ensured anonymity by using only identification numbers to label the specimens.

Policies regarding the storage of newborn blood spots vary from state to state, and storage times vary considerably. We are fortunate that Maryland provided for long-term storage. The oldest samples in our study were 14 years old at the time of retrieval, indicating the utility of this approach even for relatively "old" biological samples.

Retrieval rates were highest among nonmalformed infants (84%), but rates varied from 26% to 76% (depending on the type of cardiac anomaly) among infants with congenital heart defects. Because record matching had to be done manually (the DHMH files were computerized only after 1990) and because the specimens were organized by date of laboratory analysis, the chances of finding blood spots were diminished for infants whose blood was not drawn on the same date as others in the same nursery. Observer bias was unlikely because the collection of blood spots occurred with case–control status masked.

Several other factors appeared to influence retrieval rates. Prematurity and low birth weight reduced the retrieval rates among both cases and controls, as did early mortality among the cases. Maryland law mandates that blood spots be collected after a newborn infant has had 24 hours of milk feedings but before discharge from the hospital (2–3 days of age in 1981–1989), but allows up to 15 days to collect blood spots from sick or premature infants. We speculate that delays in oral feeding or the transfer of sick infants from community hospitals to tertiary care centers may have delayed or prevented the newborn blood screening protocol in some of our subjects. Supporting this view, Strobel and Keller [1993] reported that newborn screening of the neonatal intensive care unit population in Maryland was delayed or confounded by unavailability of a parent or guardian to sign the consent form, inadequate milk intake, and early interventions such as antibiotic administration and blood transfusion. In addition, a recent review of Maryland's newborn screening program reported problems associated with early hospital discharge of newborn infants and the resulting loss of positive predictive value of abnormal screening results [Panny, 1995].

Few studies on blood spot retrieval have been reported. In a case–control study of infants in Maryland who have oral clefts, Hwang et al. [1995] located blood spots on 75% of subjects born between 1984 and 1992. As in our study, retrieval of blood spots was lowest among seriously ill or growth-retarded infants. A descriptive study of newborn screening in London [Streetly et al., 1994] reported that, unlike our results, blood spots were less likely to be collected from African ethnic groups than from white infants and that completeness of screening varied by geographic area.

Our results demonstrate that for relatively "mild" birth defects, such as atrial septal defect, retrieval of stored blood spots is feasible using the collection process we applied in Maryland. For more severe conditions, such as truncus arteriosus and interrupted aortic arch, these methods are inadequate to provide a reasonable sample of blood spots but probably could be improved by tracing the blood spots through multiple sources, including neonatal intensive care unit databases. We recommend that blood spots be obtained routinely on admission to the intensive care nursery, as suggested by Strobel and Keller [1993], especially on seriously ill newborn infants and transferred patients. This practice was implemented in Maryland in 1991 to improve the completeness of newborn screening and will subsequently benefit future research.

ACKNOWLEDGMENTS

We thank Drs. Charlotte Ferencz, Terri Beaty, Susan Panny, and Judith Rubin for their support and guidance

and for their critical comments on the manuscript; Sheila Gordon for her kind assistance at the Maryland Department of Health and Mental Hygiene; Betty Williams for database management; and David Kauffman and Daniel Breazeale for their help in abstracting data and collecting blood spots. We especially thank Dr. Shih-Jen Hwang who developed the blood spot retrieval method and helped us to collect the samples.

REFERENCES

American College of Medical Genetics Storage of Genetics Materials Committee (1995): Statement on storage and use of genetic materials. Am J Hum Genet 57:1499–1500.

Clayton EW, Steinberg KK, Khoury MJ, Thomson E, Andrews L, Kahn JE, Kopelman LM, Weiss JO (1995): Consensus statement: Informed consent for genetic research on stored tissue samples. JAMA 274:1786–1792.

Ferencz C, Rubin JR, Loffredo CA, Magee CA (1993): "Epidemiology of Congenital Heart Disease: The Baltimore-Washington Infant Study, 1981–1989." Mt. Kisco, NY: Futura.

Hwang SJ, Beaty TH, Panny SR, Street NA, Joseph JM, Gordon S, McIntosh I, Francomano CA (1995): Association study of transforming growth factor alpha (TGFa) *Taq* I polymorphism and oral clefts: Indication of gene-environment interaction in a population-based sample of infants with birth defects. Am J Epidemiol 141: 629–636.

McCabe ERB (1991): Utility of PCR for DNA analysis from dried blood spots on filter paper blotters. PCR Methods Appl 1:99–106.

McEwen JE, Reilly PR (1994): Stored Guthrie cards as DNA "banks." Am J Hum Genet 55:196–200.

Panny SR (1995): Problems associated with early discharge by the Maryland Newborn Screening Program: Effectiveness and limitations of voluntary repeat screening. In Pass KA, Levy HL (eds): "Proceedings of the Impact of Early Discharge on Newborn Screening." Washington, DC: Association of State Public Health and Laboratory Directors, pp. 213–232.

Streetly A, Grant C, Bickler G, Eldridge P, Bird S, Griffiths W (1994): Variation in coverage by ethnic group of neonatal (Guthrie) screening programme in south London. Br Med J 309:372–374.

Strobel E, Keller CS (1993): Metabolic screening in the NICU population: A proposal for change. Pediatr Nurs 19:113–117.